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Prevalence of Virulence Factors Among Hemolytic *Escherichia coli*

Abstract

A polymerase chain reaction (PCR) assay was used to characterize 309 hemolytic *E. coli* isolates. The isolates were obtained from swine specimens presented to the diagnostic laboratory between August of 1996 and August of 1997. About one-half of the isolates contained genes for enterotoxin and/or Shiga toxin. Enterotoxigenic *E. coli* (ETEC), which cause diarrhea, were much more prevalent than Shigatoxigenic *E. coli* (STEC), which cause edema disease. K88 was the most common pilus type among ETEC and F18 was the only pilus type identified among STEC. These data are consistent with the notion that *E. coli* induced diarrheal disease is more prevalent than edema disease. However, they demonstrate that STEC persist in the swine population in spite of the low prevalence of clinical edema disease in recent years. The data suggest that vaccination and vaccine development based on K88 and F18 pilus antigens continue to be relevant for hemolytic *E. coli* infections. Some of the isolates that did not have genes for either enterotoxin or Shiga toxin, had genes for K88 or F18 pili. Such nontoxigenic isolates (NTEC) are probably not pathogenic and were speculated to act as naturally occurring K88 and F18 vaccines in some herds.

Keywords

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Prevalence of Virulence Factors Among Hemolytic *Escherichia coli*

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Summary and Implication

A polymerase chain reaction (PCR) assay was used to characterize 309 hemolytic *E. coli* isolates. The isolates were obtained from swine specimens presented to the diagnostic laboratory between August of 1996 and August of 1997. About one-half of the isolates contained genes for enterotoxin and/or Shiga toxin. Enterotoxigenic *E. coli* (ETEC), which cause diarrhea, were much more prevalent than Shigatoxigenic *E. coli* (STEC), which cause edema disease. K88 was the most common pilus type among ETEC and F18 was the only pilus type identified among STEC. These data are consistent with the notion that *E. coli* induced diarrheal disease is more prevalent than edema disease. However, they demonstrate that STEC persist in the swine population in spite of the low prevalence of clinical edema disease in recent years. The data suggest that vaccination and vaccine development based on K88 and F18 pilus antigens continue to be relevant for hemolytic *E. coli* infections. Some of the isolates that did not have genes for either enterotoxin or Shiga toxin, had genes for K88 or F18 pili. Such non-toxigenic isolates (NTEC) are probably not pathogenic and were speculated to act as naturally occurring K88 and F18 vaccines in some herds.

Introduction

Many hemolytic *E. coli* strains produce specific virulence factors that enable them to cause diarrhea or edema disease. These virulence factors are: (1) pili that mediate adhesion to the epithelium thereby allowing the *E. coli* to intensively colonize the small intestine; (2) enterotoxins that stimulate the small intestine to secrete electrolytes and water, resulting in diarrhea; and (3) Shiga toxin that is absorbed into the blood and causes systemic vascular damage resulting in edema disease. Deaths due to enterotoxigenic *E. coli* (ETEC) infections are usually the

result of diarrhea. Deaths due to Shiga toxin-producing *E. coli* (STEC) are thought to result from edema of the brain. Hemolytic activity is commonly used as a diagnostic marker for virulence of *E. coli*.

The objectives of this study were to determine: (1) what proportion of hemolytic *E. coli* isolates from swine specimens submitted to the diagnostic laboratory contained genes for pili and toxins characteristic of porcine ETEC and STEC; and (2) the comparative prevalences of different types of enterotoxins and pili among such isolates.

Materials and Methods

E. coli isolates. Hemolytic (bovine blood) *E. coli* isolates recovered from swine intestinal or fecal samples presented to the Veterinary Diagnostic Laboratory at Iowa State University from August 1996 to August 1997, were assayed for virulence factors.

PCR assay. A recently developed multiplex polymerase chain reaction (PCR) assay (1) was used to determine if the isolates carried genes for heat labile *E. coli* enterotoxin (LT), heat stable *E. coli* enterotoxins of the (STa) and (STb) types, Shiga toxin type 2e (Stx2e), and K88, F18, K99, 987P, and F41 pili.

Results and Discussion

The results are summarized in Table 1. Of the 309, isolates, 49% had genes for enterotoxin and/or Shiga toxin and were classified as ETEC or STEC. Most of the ETEC had genes for more than one type of enterotoxin, with STb and LT being the most prevalent. K88 was the most prevalent pilus type, followed by F18. The comparatively low prevalence of the other pilus types is probably because K99, 987P and F41 are usually associated with non-hemolytic ETEC. Presumably the six ETEC isolates that did not carry genes for any of the pili would not have been able to colonize the small intestine and therefore were nonpathogenic. Alternatively, they may have carried genes for other (new or as yet unrecognized) pilus types that mediate colonization of pig small intestine.

ETEC were more than 10 times as prevalent as STEC. This is consistent with recent clinical experience indicating that diarrhea attributed to *E. coli* continues to be a common problem, whereas edema disease is infrequently encountered. The occurrence of six isolates with genes for both Stx2e and F18 pili suggests that edema disease pathogens are still present in the swine population in spite of the comparatively low incidence of edema disease among U.S. swine for the last two decades.

Half of the isolates (51%) were classified as nontoxigenic *E. coli* (NTEC), and were presumably not

pathogenic. Several of the NTEC had genes for F18 or K88 pili. F18 was as prevalent among the NTEC isolates as it was among toxigenic isolates. We speculate that these F18⁺ and K88⁺ NTEC may act as naturally occurring immunogens (pilus vaccines) protecting against diarrhea and edema disease in some herds (2,3).

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Table 1. Virulence genes detected by polymerase chain reaction assay of hemolytic *Escherichia coli* isolates from swine.

Escherichia coli Isolates from Swine											
E. coli		Virulence Genes									
Pathotype	No. of Isolates	Enterotoxins*			Shiga Toxin	Pili **					
		LT	STa	STb	Stx2e	K88	F18	K99	987P	F41	None
ETEC	138	101	47	130	0	112	18	1	1	4	6
STEC	12	0	4***	4	12	0	6	0	0	0	6
NTEC	159	0	0	0	0	5	25	0	0	0	128
Total	309	101	51	134	12	117	49	1	1	4	140

* Most ETEC had genes for two or three different enterotoxins.

** Several isolates had genes for two pilus types.

*** Four isolates had genes for enterotoxins and Shiga toxin but are listed only as STEC.